

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (original): A method of detecting an analyte comprising the steps of:

- (a) anchoring said analyte to a nucleic acid template;
- (b) conducting a nucleic acid polymerase reaction to produce labeled polyphosphate, said reaction comprising the reaction of said template, a primer, at least one terminal phosphate-labeled nucleotide, and a nucleic acid polymerase; and
- (c) analyzing said labeled polyphosphate.

Claim 2 (original): The method of claim 1, wherein said primer is a nuclease resistant primer.

Claim 3 (original): The method of claim 2, wherein the nucleic acid polymerase reaction further includes an enzyme having 3' → 5' exonuclease activity.

Claim 4 (original): The method of claim 1, wherein said analyzing step includes (a) reacting said labeled polyphosphate with a phosphatase to produce a detectable species characteristic of said analyte and (b) detecting said detectable species.

Claim 5 (original): The method of claim 1, further including the step of separating any nucleic acid template not anchored by said analyte before said conducting step.

Claim 6 (currently amended): The method of claim 4, wherein said at least one terminal phosphate-labeled nucleotide is substantially non-reactive to phosphatase, further wherein said reacting step and said conducting step are carried out simultaneously.

Claim 7 (original): The method of claim 1, further comprising the step of characterizing said analyte.

Claim 8 (original): The method of claim 7, further comprising the step of quantifying said analyte.

Claim 9 (original): The method of claim 1, wherein said analyte is DNA, RNA, protein, lipid, oligosaccharide, a whole cell, or a synthetic polymer.

Claim 10 (original): The method of claim 1, wherein said analyte is anchored to said nucleic acid template by non-covalent binding, or by one or more covalent bonds.

Claim 11 (original): The method of claim 1, wherein said nucleic acid polymerase is a DNA polymerase or an RNA polymerase.

Claim 12 (original): The method of claim 2, wherein said nuclease resistant primer includes a methyl phosphonate, a borano phosphate or a phosphorothioate linkage.

Claim 13 (original): The method of claim 1, wherein said nucleic acid template and said primer are switched and it is said primer that is anchored to the analyte.

Claim 14 (original): The method of claim 1, wherein said nucleic acid template and said primer are part of a DNA hairpin, and said DNA hairpin is anchored to said analyte in said anchoring step.

Claim 15 (original): The method of claim 4, wherein said detectable species is detectable by a property selected from the group consisting of color, fluorescence emission, chemiluminescence, mass change, oxidation/reduction potential and combinations thereof.

Claim 16 (original): The method of claim 4, wherein said detectable species is produced in amounts substantially proportional to the amount of analyte.

Claim 17 (original): The method of claim 1, wherein at least one terminal phosphate-labeled nucleotide includes four or more phosphate groups in the polyphosphate chain.

Claim 18 (original): The method of claim 1, wherein the labels in at least one terminal phosphate-labeled nucleotide are enzyme-activatable labels selected from the group consisting of chemiluminescent compounds, fluorogenic dyes, chromogenic dyes, mass tags, electrochemical tags and combinations thereof.

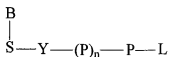
Claim 19 (original): The method of claim 1, wherein said terminal phosphate-labeled nucleotides carry distinct labels.

Claim 20 (original): The method of claim 19, wherein the presence of an analyte is determined by the ratio of distinct labels produced.

Claim 21 (original): The method of claim 1, wherein one or more additional detection reagents are added in said polymerase reaction of said conducting step, and said additional detection reagents are capable of a response that is detectably different from said labeled polyphosphate.

Claim 22 (original): The method of claim 1, wherein at least one terminal phosphate-labeled nucleotides are deoxy nucleotides and carry different labels.

Claim 23 (original): The method of claim 1, wherein at least one terminal-phosphate-labeled nucleotide is represented by the formula:



wherein P is phosphate (PO_3) and derivatives thereof, n is 2 or greater; Y is an oxygen or sulfur atom; B is a nitrogen-containing heterocyclic base; S is an acyclic moiety, carbocyclic moiety or sugar moiety; L is an enzyme-activatable label containing a hydroxyl group, a sulfhydryl group or an amino group suitable for forming a phosphate ester, a thioester or a phosphoramidate linkage at the terminal phosphate of a natural or modified nucleotide; and P-L is a phosphorylated label which preferably becomes independently detectable when the phosphate is removed.

Claim 24 (original): The method of claim 23, wherein said sugar moiety is selected from the group consisting of ribosyl, 2'-deoxyribosyl, 3'-deoxyribosyl, 2', 3'-dideoxyribosyl, 2', 3'-didehydrodideoxyribosyl, 2'-alkoxyribosyl, 2'-azidoribosyl, 2'-aminoribosyl, 2'-

fluororibosyl, 2'-mercaptoriboxyl, 2'-alkylthioribosyl, carbocyclic, acyclic and other modified sugars.

Claim 25 (original): The method of claim 23, wherein said base is selected from the group consisting of uracil, thymine, cytosine, guanine, 7-deazaguanine, hypoxanthine, 7-deazahypoxanthine, adenine, 7-deazaadenine, 2,6-diaminopurine and analogs thereof.

Claim 26 (original): The method of claim 23, wherein said enzyme-activatable label is selected from the group consisting of chemiluminescent compounds, fluorogenic dyes, chromogenic dyes, mass tags, electrochemical tags and combinations thereof.

Claim 27 (original): The method of claim 26, wherein said enzyme-activatable label is a fluorogenic moiety selected from the group consisting of 2-(5'-chloro-2'-phosphoryloxyphenyl)-6-chloro-4-(3H)-quinazolinone, fluorescein diphosphate, fluorescein 3'-(6')-O-alkyl-6'-(3')-phosphate, 9H-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl)phosphate, 4-methylumbelliferyl phosphate, resorufin phosphate, 4-trifluoromethylumbelliferyl phosphate, umbelliferyl phosphate, 3-cyanoumbelliferyl phosphate, 9,9-dimethylacridin-2-one-7-yl phosphate, 6,8-difluoro-4-methylumbelliferyl phosphate, and derivatives thereof.

Claim 28 (original): The method of claim 26, wherein said phosphorylated label is a chromogenic moiety selected from the group consisting of 5-bromo-4-chloro-3-indolyl phosphate, 3-indoxyl phosphate, *p*-nitrophenyl phosphate, and derivatives thereof.

Claim 29 (original): The method of claim 26, wherein said chemiluminescent compound is an alkaline phosphatase-activated 1,2-dioxetane compound.

Claim 30 (original): The method of claim 29, wherein said 1,2-dioxetane compound is selected from the group consisting of 2-chloro-5-(4-methoxyspiro[1,2-dioxetane-3,2'-(5-chloro)-tricyclo[3,3,1-1^{3,7}]-decan]-1-yl)-1-phenyl phosphate, chloroadamant-2'-ylidenemethoxyphenoxy phosphorylated dioxetane, 3-(2'-spiroadamantane)-4-methoxy-4-(3''-phosphoryloxy)phenyl-1,2-dioxetane and derivatives thereof.

Claim 31 (withdrawn): A kit for detecting an analyte comprising:

- (a) at least one terminal-phosphate-labeled nucleotide;
- (b) a DNA polymerase; and
- (c) a phosphatase.

Claim 32 (withdrawn): A kit for detecting an analyte according to claim 31, further comprising: a nuclease with enzymatic activity sufficient to decompose DNA in the 3' → 5' direction.

Claim 33 (withdrawn): A kit for detecting an analyte according to claim 31, wherein said DNA polymerase has nuclease activity sufficient to decompose DNA in the 3' → 5' direction.

Claim 34 (withdrawn): A kit for detecting an analyte according to claim 31, further comprising:

- (a) at least one nucleic acid template; and
- (b) at least one nuclease resistant primer complementary to said at least one nucleic acid template;

wherein said at least one nucleic acid template and/or said complementary nuclease resistant primer has an anchoring moiety.

Claim 35 (withdrawn): A kit for detecting an analyte according to claim 31, further comprising at least one hairpin template-primer combination with a nuclease resistant 3'-end.

Claim 36 (original): A method of detecting and characterizing multiple analytes in a sample, comprising the steps of:

- (a) anchoring to each analyte a specific template nucleic acid sequence with a unique base at the site opposite to the complementary nucleotide being added;

- (b) conducting a DNA polymerase reaction to produce labeled polyphosphates, said reaction comprising the reaction of said templates, primers complementary to said specific template sequence, two or more terminal phosphate-labeled nucleotides with different labels, a DNA polymerase and an enzyme having $3' \rightarrow 5'$ exonuclease activity;
- (c) permitting said labeled polyphosphates to react with a phosphatase to produce detectable species unique to each of said analytes; and
- (d) detecting said detectable species.

Claim 37 (original): A method of detecting and characterizing multiple analytes in a sample, comprising the steps of:

- (a) anchoring to each analyte a specific template nucleic acid sequence with a unique base at the site opposite to the complementary nucleotide being added;
- (b) conducting a DNA polymerase reaction to produce uniquely labeled polyphosphates; said reaction comprising the reaction of said templates, nuclease resistant primers complementary to said specific target sequence of each of said multiple analytes, two or more terminal phosphate-labeled nucleotides having 4 or more phosphate groups in the polyphosphate chain and each bearing a different label, a DNA polymerase and an enzyme having $3' \rightarrow 5'$ exonuclease activity; and
- (c) detecting the labeled polyphosphates.

Claim 38 (original): A method of detecting and characterizing multiple analytes in a reaction compartment, comprising the steps of:

- (a) anchoring a unique template nucleic acid sequence to each of said analytes;
- (b) anchoring said analytes to the surface of said reaction compartment;
- (c) conducting a DNA polymerase reaction to produce labeled polyphosphate; said reaction comprising the reaction of the unique template sequence of one of said analytes, a nuclease resistant primer complementary to said unique template sequence, at least one terminal phosphate-labeled nucleotides having 4 or more phosphate groups in the polyphosphate chain, a DNA polymerase and an enzyme having 3' → 5' exonuclease activity;
- (d) detecting said labeled polyphosphate;
- (e) washing off the unanchored components; and
- (f) repeating steps (a) to (d) with a nuclease resistant primer complementary to another unique template sequence of a different analyte until all the analytes are analyzed.

Claim 39 (original): The method of claim 38, wherein said at least one terminal phosphate labeled nucleotides have 4 or more phosphate groups in the polyphosphate chain.

Claim 40 (original): The method of claim 38, wherein said detecting step includes:

- (a) permitting said labeled polyphosphate to react with a phosphatase to produce a detectable species; and
- (b) detecting said detectable species.